

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY¹]Steroidal Sapogenins. XLIX. C-Ring Oxygenated Derivatives of Correllogenin²

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Received May 19, 1958

In the course of conversion of natural mixtures of the 25D and 25L isomeric sapogenins gentrogenin (25D) and correllogenin (25L) to 11-keto diosgenin and 11-keto yamogenin, it was found possible to separate and characterize several intermediates which were sterically pure 25L compounds, viz. 3 β ,12 β -dihydroxy-20 α ,22 β ,25L-spirost-5-en-11-one, Ia, and its diacetate, Ib; 20 α ,22 β ,25L-spirost-5-en-3 β ,11 α -diol, IIa, and its diacetate, IIb. These new correllogenin derivatives have previously evaded isolation because of the difficulties in separating the pure parent compound. It was found that 3 β ,12 β -diacetoxy-20 α ,22 β ,25D-spirost-5-en-11-one on treatment with calcium in liquid ammonia solution gave, in the presence of water, a high yield of 20 α ,22 β ,25D-spirost-5-en-3 β ,11 α -diol (11 α -hydroxy diosgenin).

In earlier papers of this series^{3,4} we have described the isolation of gentrogenin³ (3 β -hydroxy-20 α ,22 β ,25D-spirost-5-en-12-one⁵), its 25L-diastereoisomer, correllogenin,³ and the conversion of the former to 11-keto diosgenin.⁴ In the latter paper

were described the properties and reactions of a number of C-11 and C-12 oxygenated derivatives of gentrogenin. Because of the unavailability of pure correllogenin,⁶ we were unable to prepare the corresponding 25L- derivatives. During the large scale conversion of a gentrogenin-correllogenin mixture to 3 β -hydroxy-5,16-pregnadiene-11,20-dione,⁷ we were able to isolate and characterize

(1) Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

(2) Paper XLVIII, E. S. Rothman and M. E. Wall, submitted to *J. Am. Chem. Soc.*

(3) H. A. Walens, S. Serota, and M. E. Wall, *J. Org. Chem.*, **22**, 182 (1957).

(4) E. S. Rothman and M. E. Wall, *J. Am. Chem. Soc.*, **79**, 3228 (1957).

(5) For basis of formal nomenclature, particularly at C₂₂, cf. *Tentative Rules for Steroid Nomenclature*, Comptes Rendus de la Dix-Huitieme Conference, Zurich, 20-28 July, 1955, pp. 190-198.

(6) Although correllogenin may constitute twenty per cent of the total ketonic fraction isolated from *D. spiculiflora*, it is difficult to separate this sapogenin from the isomeric gentrogenin³ and consequently only minute quantities of the sterically pure 25L- form have ever been obtained.

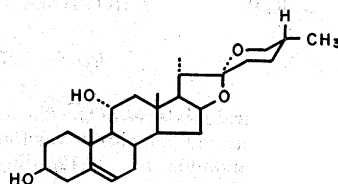
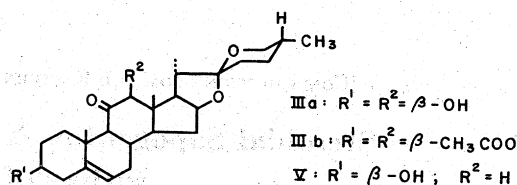
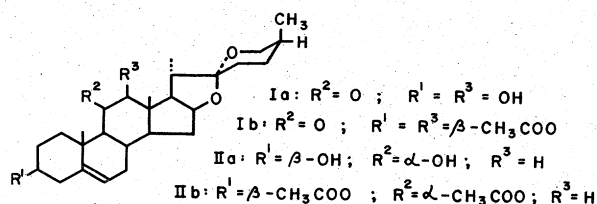
(7) Since pseudomerization followed by oxidative cleavage and alkaline hydrolysis converts the 25D- and 25L-sapogenins to the same 16-dehydro-20-keto-pregnene it is often convenient to work directly with the mixture.

several sterically pure corrollogenin derivatives. In this paper we wish to record the properties of these compounds as well as those of the corresponding 25D- series.

In the manner described previously,⁴ bromination of a gentrogenin-corrollogenin mixture gave the 5 α ,6 β ,11 α ,23-tetrabromo-sapogenins which, on treatment with sodium iodide, gave the 11 α ,23-dibromo sapogenins of the 25D- and 25L- mixture. Alkaline equilibration in aqueous tertiary butyl alcohol⁸ gave a mixture of the isomeric 25D- and 25L- derivatives of 3 β ,12 β -diacetoxy-20 α , 22 β -spirosten-5-en-11-one. In experiments in the hecogenin series,⁸ the English workers found that alkaline equilibration of 11 α ,23-dibromohecogenin acetate in methanol or ethanol gave, in addition to the desired 12 β -hydroxy-11-one, some of the undesirable 11-hydroxy-12-ones. In contrast, similar equilibration in hot aqueous *t*-butyl alcohol gave only the desired 12 β -hydroxy-11-one. We also⁴ had previously established the absence of 12 ketone in the equilibrium mixture (by testing with Girard's reagent) when the hydrolysis step was carried out in the case of the sterically pure, brominated gentrogenin derivative. We then saponified and equilibrated a mixture of brominated sapogenins derived from a natural gentrogenin-corrollogenin mixture in the tertiary-butanol system and, reasoning by analogy, felt that such treatment would lead to the desired 12 β -hydroxy-11-one. After a series of crystallizations and treatment with carbon (cf. Experimental section) a residual fraction was saponified, resulting in the isolation of a small quantity of the very insoluble, sterically pure 3 β ,12 β -dihydroxy-20 α ,22 β ,25L-spirost-5-en-11-one (Ia). Acetylation in hot acetic anhydride-pyridine gave 3 β ,12 β -diacetoxy-20 α ,22 β ,25L-spirost-5-en-11-one (Ib). Because the yield of Ia was less than one per cent of the total starting material it was necessary to prove its structure. The infrared spectra of Ia and Ib showed the typical "normal" (25L) series of bands in the 800-1000 cm.⁻¹ region.⁹ The spectrum of Ib showed the same acetate band shift from 1735 cm.⁻¹ to 1750 cm.⁻¹ observed in 11,12-ketol derivatives in the hecogenin series.⁸ The carbon and hydrogen analysis of Ib confirmed the tentative structural assignments. Compound Ia did not react with Girard's reagent T eliminating from consideration ketols with the 11-hydroxy-12-one moiety. That the structure of Ia and Ib was indeed that of a 12 β -hydroxy-11-one and the corresponding acetate, respectively, was shown in a clear-cut manner by the molecular rotation contribution of the 12-acetoxy group. It was shown in the hecogenin series that the Δ -M_D for a 12 β -acetoxy-11-one was -237° and for the correspond-

ing 12 α -acetoxy-11-one, +212.⁸ The corresponding value for Ib was -226° \pm 50.¹⁰ Hence the structure of Ia and Ib is indeed in accord with our predictions based on analogy.

The remaining crystalline fractions (after removal of sterically pure Ia) were combined, reacylated, and reduced with calcium-ammonia¹¹ as described previously.⁴ Chromatography of the saponified product on Florisil¹² gave first the expected mixture of 11-keto diosgenin and 11-keto yamogenin. A more tenaciously adsorbed fraction was then eluted from which, after reacylation followed by several recrystallizations, we obtained an appreciable quantity of 3 β ,11 α -diacetoxy-20 α ,22 β ,25L-spirost-5-ene (IIb). Saponification gave 20 α ,22 β ,25L-spirost-5-ene-3 β ,11 α -diol (IIa).



The structure proof of IIa and IIb is based on the following considerations. The infrared spectrum of IIa shows the typical "normal" (25L) sapogenin fingerprint bands⁹ and absence of carbonyl. Carbon and hydrogen analyses of IIa and IIb are in

(10) In order to calculate the M_D contribution of the 12 acetoxy group in Ib it was necessary that the M_D of the corresponding 12-desoxy steroid, 11-keto yamogenin acetate be known. Since the latter has not been prepared it was necessary to assign a hypothetical value based on the following specific rotations:

12-keto diosgenin acetate (25D) = -56°

12-keto yamogenin acetate (25L) = -60°

11-keto diosgenin acetate (25D) = -86°

hence 11-keto yamogenin acetate (25L) = -90° \pm 10°

The $\pm 10^\circ$ factor is well within the differences between all known pairs of 25D and 25L diastereoisomers.

(11) J. H. Chapman, J. Elks, G. H. Phillips, and L. J. Wyman, *J. Chem. Soc.*, 4344 (1956).

(12) Mention of commercial products does not imply endorsement by the U. S. Department of Agriculture over similar products not mentioned.

(8) J. Elks, G. H. Phillips, T. Walker, and L. J. Wyman, *J. Chem. Soc.*, 4330 (1956).

(9) M. E. Wall, C. R. Eddy, M. L. McClellan, and M. E. Klumpp, *Anal. Chem.*, 24, 1337 (1952).

accord with the formulation of a dihydroxy or diacetoxysapogenin, respectively. From the method of preparation the compounds must have a $3\beta,11$ -dihydroxy function. Since IIb is a diacetate produced under mild acetylation conditions IIa and IIb must have, respectively, the $3\beta,11\alpha$ -dihydroxy and $3\beta,11\alpha$ -diacetoxysapogenin functions. The hitherto unknown $3\beta,12\beta$ -dihydroxy- $20\alpha,22\beta,25$ -spirost-5-en-11-one (IIIa) was prepared by saponification of the known⁴ $3\beta,12\beta$ -diacetate (IIIb).

Previously,⁵ we had prepared $20\alpha,22\beta,25$ -spirost-5-ene- $3\beta,11\alpha$ -diol (IV) in low yield by reduction of $3\beta,12\beta$ -diacetoxysapogenin- $20\alpha,22\beta,25$ -spirost-5-en-11-one (IIIb) with calcium-anhydrous liquid ammonia, the chief product being the corresponding 11-ketone (V). Reduction of IIIb with calcium-liquid ammonia in the presence of water gave IV as the sole product in good yield.

The reductive deacetoxylation of a non-olefinic 12β -acetoxysapogenin-11-ketone with calcium in ammonia to the point of disappearance of the blue calcium solution color followed by the addition of methanol was reported by Chapman, Elks, Philipps, and Wyman.¹¹ Their product was 11α -hydroxy tigenin. The behavior of a Δ^5 - 3β -hydroxy system toward such a reactive reagent is not predictable. Birch¹³ has noted that the presence of protonic substances, for example water, profoundly affects the course of such reductions. In such systems benzene forms 1,4-dihydrobenzene and allyl alcohols are converted to hydrocarbons. While Δ^5 - 3β -ols are not true allyl alcohols, they have many properties that suggest a close interrelationship between the olefin and alcohol groups. For example, 3,5-cyclo systems form under appropriate conditions. When we reduced compound IIIb with blue calcium-liquid ammonia-water systems we did not observe any attack on the Δ^5 - 3β -hydroxy system and obtained compound IV directly in high yield.

EXPERIMENTAL

$3\beta,12\beta$ -Dihydroxy- $20\alpha,22\beta,25$ -spirost-5-en-11-one (Ia). The isolation of Ia was fortuitous. We had been working up mother liquors from a debromination experiment leading to 160 g. of the isomeric mixture of $3\beta,12\beta$ -diacetoxysapogenin- $20\alpha,22\beta$ -spirost-5-en-11-one compounds.⁴ The first crop from 300 ml. of ethanol was 69 g. of 25d and 25l mixed product. The filtrate was diluted with 300 ml. of benzene and then with 1700 ml. of petroleum ether, b.p. 89–98°. The clear supernatant liquor was decanted from 64 g. of a tarry residue (which later yielded an additional 21 g. of crystalline mixed product) and the solution was boiled to expel benzene. The cooled filtrate was treated with 100 g. of Darco G-60 carbon which was well washed with petroleum ether. These washings were concentrated to a small volume whereupon 8.2 g. of crystalline mixed isomers separated. Further washing of the carbon with methylene chloride and with ethanol gave, after evaporation, 17 g. of a glassy residue. Saponification of the residue gave 1.03 g. of a very insoluble crystalline, m.p. 244–247°, insoluble in methylene chloride and in ethanol but soluble in a 1:1 mixture of these solvents.

The product recrystallized from this mixture in hexagonal prisms, $[\alpha]_D^{25}$ -112° , and melted from 247 to 248° with characteristic transition to smaller, bladed forms only above 240°. The infrared spectrum (KBr disk) showed a strong hydroxyl band envelope at 3400 to 3500 cm^{-1} , ketone at 1716 cm^{-1} and sapogenin bands at 846, 900, 917, and 985 cm^{-1} of the type characteristic of 25l sapogenins. The fingerprint spectrum was highly complex showing forty-five well-defined bands. In chloroform solution the ketone band occurred at 1707 cm^{-1} .

$3\beta,12\beta$ -Diacetoxysapogenin- $20\alpha,22\beta,25$ -spirost-5-en-11-one (Ib). The dihydroxy ketone of the above preparation was acetylated by refluxing for 2 hr. in 1:1 acetic anhydride-pyridine mixture (forcing conditions). After cooling and diluting with water, the steroid was collected by filtration and dried. Its solution in methylene chloride was freed of brownish coloration by passing through a pad of Florisil.¹² After evaporation to dryness the colorless residue was crystallized from ethanol to give felted microneedles, $[\alpha]_D^{25}$ -120° , m.p. 194.3–196.8°, after slight sweating at 178°.

Anal. Calcd. for $\text{C}_{31}\text{H}_{48}\text{O}_7$: C, 70.43; H, 8.39. Found: C, 70.48; H, 8.66.

$3\beta,11\alpha$ -Diacetoxysapogenin- $20\alpha,22\beta,25$ -spirost-5-en-11-one (IIb). Ninety grams of a C-25 diastereoisomeric mixture of $3\beta,12\beta$ -diacetoxysapogenin- $20\alpha,22\beta$ -spirost-5-en-11-one was deacetoxylation by reduction with calcium in liquid ammonia.⁴ Chromatography of the saponified reduction product on Florisil¹² gave, on elution with 20% chloroform in benzene, the expected mixture of 11-keto diosgenin and 11-keto yamogenin. Further elution with chloroform gave a dihydroxy fraction only partly soluble in benzene. The benzene suspension was filtered and the filtrate, which was richer in the 25l-component, was evaporated to dryness. The residue was crystallized from ether and acetylated to give 10 g. of the nearly sterically pure isomer $3\beta,11\alpha$ -diacetoxysapogenin- $20\alpha,22\beta,25$ -spirost-5-en-11-one (IIb). A single recrystallization from methanol gave a sterically pure sample. Five recrystallizations from methanol did not alter the melting point, viz. 190–191.5°, $[\alpha]_D^{25}$ -123° , feathery, felted microneedles.

Anal. Calcd. for $\text{C}_{31}\text{H}_{46}\text{O}_6$: C, 72.34; H, 9.01. Found: C, 72.62; H, 9.07.

$20\alpha,22\beta,25$ -Spirost-5-en- $3\beta,11\alpha$ -diol (IIa). A sample of the diacetate of the preceding preparation was saponified in methanolic 5% potassium hydroxide. Crystallization from methylene chloride-hexane gave hexagonal prisms with pyramidal caps $[\alpha]_D^{25}$ -123° , m.p. 247.2–248.2° after transition over 243° to wedges.

Anal. Calcd. for $\text{C}_{27}\text{H}_{42}\text{O}_4$: C, 75.31; H, 9.83. Found: C, 75.05; H, 9.91.

$3\beta,12\beta$ -Dihydroxy- $20\alpha,22\beta,25$ -spirost-5-en-11-one (IIIa). A sample of the diacetate⁴ was saponified by refluxing in methanolic 5% potassium hydroxide for 4 hr. The mixture was poured into water and the steroid collected. The dried product was dissolved in ether, diluted with hexane, and the solution was freed of ether by volume reduction to yield a crystalline residue which was further recrystallized from acetone to give short, hexagonal microneedles, $[\alpha]_D^{25}$ -95° , melting from 236–240° and giving a pink melt. Incomplete transition to branched filaments was observed beyond 223°.

Reduction of IIIb to $20\alpha,22\beta,25$ -spirost-5-en- $3\beta,11\alpha$ -diol (11 α -hydroxydiosgenin) (IV). A sample of $3\beta,12\beta$ -diacetoxysapogenin- $20\alpha,22\beta,25$ -spirost-5-en-11-one, 85 g., in 900 ml. of toluene was added to a solution of 67 g. of calcium metal in 4 liters of liquid ammonia during an addition time of 20 min. The mixture was mechanically stirred during the addition and during the subsequent reaction time of 10 min. Water was added cautiously in a thin stream until the blue color of the reaction mixture was discharged, an excess of water doing no harm. The mixture was evaporated in an open vessel to a white solid residue. This residue was shaken with ether and dilute aqueous hydrochloric acid until all solids were in solution. The organic layer was separated, washed with water and with saturated saline solution, and evaporated to dryness. To insure complete saponification of the 3-acetate it

(13) A. J. Birch, *Quart. Revs. (London)*, 4, 69 (1950).

was occasionally necessary to carry out a saponification step with 5% methanolic caustic. The product, m.p. 228–233°, was very soluble in hexane and ether but gave thick, hexagonal prism-like forms on recrystallization. The analytical sample thus obtained melted from 233–235° after undergoing crystal transition above 228° to whips, $[\alpha]_D^{25} -116^\circ$ (CHCl₃). This compound is 11 α hydroxy diosgenin.

Acknowledgments. The authors wish to thank S. Serota for optical rotation measurements, C. R. Eddy and C. Leander for infrared spectra, and C. L. Ogg and associates for semimicroanalyses.

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